Effect of Dietary Tryptophan on Plasma and Brain Tryptophan, Brain Serotonin, and Brain 5-Hydroxyindoleacetic Acid in Rainbow Trout

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*In order to determine the effect of dietary tryptophan level on plasma and brain tryptophan, brain serotonin, and brain 5-hydroxyindoleacetic acid levels, juvenile rainbow trout (*Salmo gairdneri*) were raised for 16 weeks on semipurified diets containing 0.06%, 0.16%, 0.21%, 0.26%, 0.39%, or 0.59% tryptophan. After 14 weeks, feed intake was depressed in fish fed the diets containing 0.06% or 0.16% tryptophan. No further differences in feed intake were noted between the remaining treatments. In addition, body weight was lower in fish fed diets containing 0.06%, 0.16%, or 0.21% tryptophan compared with fish fed higher levels. After 16 weeks of feeding the test diets, plasma tryptophan levels were found to be directly related to dietary tryptophan levels. Similarly, increased dietary levels of tryptophan resulted in increased brain levels of tryptophan, serotonin, and 5-hydroxyindoleacetic acid. These results demonstrate that in rainbow trout, as in mammals, altered dietary levels of tryptophan result in alterations in plasma and brain tryptophan, brain serotonin, and brain 5-hydroxyindoleacetic acid.*

Keywords: Rainbow trout; plasma tryptophan; brain tryptophan; serotonin; 5-hydroxytryptamine; food intake

Introduction

In mammals, increasing the level of dietary tryptophan (TRP) results in an elevation of plasma TRP.[1] This higher plasma TRP level increases the availability of TRP for uptake into the brain, resulting in elevated brain TRP levels.[2] In the biosynthesis of the neurotransmitter serotonin (5-HT) from TRP, TRP 5-monooxygenase (EC 1.14.16.4) catalyzes the rate-limiting step. However, this enzyme is not saturated at physiologic levels; therefore, elevations in brain TRP levels result in enhanced brain 5-HT synthesis.[2] In addition, the level of 5-hydroxyindoleacetic acid (5-HIAA), the principal brain metabolite of 5-HT, is also increased. These elevations in brain 5-HT and, especially, in brain 5-HIAA are believed to be indicative of increased serotonergic neurotransmission in these animals and have been found to be associated with a number of physiologic responses, including satiety.[2,3]

In rainbow trout (*Salmo gairdneri*), increased serotonergic neurotransmission resulting from altered net availability of TRP to the brain has also been proposed.[4] However, there has been no demonstration of a direct relationship between dietary TRP and plasma TRP, brain TRP, brain 5-HT, and brain 5-HIAA. Therefore, this study was conducted in order to determine the effect of dietary TRP level on the levels of plasma TRP, brain TRP, brain 5-HT, and brain 5-HIAA in rainbow trout. The dietary TRP concentrations used in this study were selected to include levels below, at, and above the TRP requirement of 0.25% reported for rainbow trout by Walton et al.[5]

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Materials and Methods

Diet preparation and analysis

Semipurified diets, modified from those used by Chance et al.,[6] were used in the experiment, with gelatin serving as the TRP-deficient protein source (*Table 1*). Casein and crystalline amino acids were added such that (with the exception of TRP) the overall amino acid profile of the diet reflected that of cod white muscle.[5] Crystalline TRP was added to formulate diets ranging from 0.06% to 0.59% TRP in order to provide diets below, at, and above the estimated TRP requirement (0.25%).[5] The remainder of the diet formulation was designed to meet the National Research Council's suggested requirements for rainbow trout.[7] Diets were then processed by mixing two parts of dry diet with one part of hot distilled water (90°C). The diets were then allowed to set to firmness (30 minutes) at 4°C before milling into feed-sized pellets using a Hobart meat grinder (Model M-802, Hobart Manufacturing Company Ltd., Don Mills, Ontario, Canada). Throughout the experiment, diets were stored at −10°C and were weighed out biweekly for feeding to the fish.

Table 1 Diet formulation and analysis[a]

	Diet					
	1	2	3	4	5	6
Ingredients						
Gelatin	30	30	30	30	30	30
Casein	5	5	5	5	5	5
Fish oil	15	15	15	15	15	15
Corn starch	14.00	13.90	13.85	13.80	13.67	13.47
Mineral premix[b]	9	9	9	9	9	9
Vitamin premix[c]	2	2	2	2	2	2
Amino acid premix[d]	25	25	25	25	25	25
L-Tryptophan	0.00	0.10	0.15	0.20	0.33	0.53
Analysis						
Moisture	34	33	34	34	34	33
Protein	56	57	56	56	56	55
Lipid	16	17	16	16	16	17
Ash	9	9	9	10	10	9
Tryptophan	0.06	0.16	0.21	0.26	0.39	0.59

[a] Ingredients and analyzed results are expressed as percent, dry matter basis (except for moisture).

[b] The mineral premix supplied the following in g/kg dry diet: $CaH_2PO_4 \cdot 2H_2O$, 30; $CaCO_3$, 2; NaCl, 15; K_2SO_4, 20; $MgSO_4$, 20; $Fe_2SO_4 \cdot 7H_2O$, 0.7; $MnSO_4 \cdot H_2O$, 0.3; $ZnSO_4 \cdot 7H_2O$, 0.85; $CuSO_4 \cdot 5H_2O$, 0.16; KI, 0.015; Na_2SeO_3, 0.0025; $CoCl_2 \cdot 6H_2O$, 0.026; and corn starch as carrier.

[c] The vitamin premix supplied the following in mg/kg dry diet (except where units are noted): thiamine mononitrate, 15; riboflavin, 50; niacin, 296; pyridoxine HCl, 40; D-calcium pantothenate, 200; folic acid, 17; biotin, 1.0; cyanocobalamin, 0.2; inositol, 500; choline chloride, 5,500; L-ascorbic acid, 1,000; butylated hydroxytoluene, 25; vitamin A (retinol palmitate and retinol acetate), 7,000 IU/kg; vitamin D_3, 3,000 IU/kg; vitamin E (dl-α-tocopherol acetate), 300; vitamin K (menadione sodium bisulphite), 50; and corn starch as carrier.

[d] The amino acid premix supplied the following in g/kg dry diet: L-arginine HCl, 6.25; L-histidine $HCl \cdot H_2O$, 11.25; L-isoleucine, 15; L-leucine, 28; L-lysine HCl, 31.25; DL-methionine, 4.75; L-cyst(e)ine, 6.5; L-phenylalanine, 11.75; L-tyrosine (disodium salt), 13; L-threonine, 15.5; L-valine, 19.75; L-alanine, 3; L-aspartic acid, 6.75; L-glutamic acid (monosodium salt), 38.75; L-serine, 16.75; and corn starch as carrier.

Dietary TRP was analyzed by Woodson-Tenent Laboratories, Inc. (Goldston, NC, USA) using high-performance liquid chromatography (HPLC) following alkaline hydrolysis. Samples were dried at 100°C for 16 hours to determine dietary moisture, and were ashed by combusting at 600°C for 16 hours. Crude protein was determined by Kjeldahl N analysis using a Tecator Kjeltec Auto 1030 Analyzer (Fisher, Toronto, Ontario, Canada) and lipid was determined by the method of Bligh and Dyer.[8] A summary of the diet analysis appears in *Table 1*.

Supply and maintenance of fish

Juvenile rainbow trout (each weighing approximately 3 g) were obtained from Spring Valley Fish Farm (Petersburg, Ontario, Canada) and were acclimated for 4 weeks in a shallow fry trough. During this time, they were fed a commercial salmonid starter diet (Martin Feed Mills, Elmira, Ontario, Canada). Seventy fish per tank were then randomly distributed to 18 dark-green, 40-L fiberglass tanks (initial body weight, 237 g/tank ± 3.2%). The diets were assigned to the 18 tanks in a randomized complete block design with three replicates (tanks) per treatment. Fish were fed 4 times/day, 7 days/week to satiety, and were weighed at the end of 4, 8, 12, and 14 weeks. Throughout the experiment, the following environmental conditions were maintained (expressed as mean, with ranges in parentheses): dissolved oxygen, 7.6 ppm (6.3–8.3); ammonia nitrogen, 0.65 ppm (0.60–0.80); flow rate, 3.8 L/min (3.6–4.0); temperature, 14.8°C (13.0–16.0); and pH, 6.6 (6.5–6.8).

Sampling of fish and tissue analysis

After 16 weeks, fish fed 0.06% TRP were too small to yield blood samples. However, 12 fish were removed from all other tanks and immediately anesthetized in a solution of tricaine methane sulfonate. Pooled blood was collected in heparinized tubes by tail amputation at the caudal peduncle of 12 fish to provide three samples per tank (four fish per sample). The blood was centrifuged at 5,000 × *g* for 5 minutes, and the resultant plasma was frozen at −80°C for 7 weeks until analysis of TRP. Immediately following blood collection, brains were removed from the fish and frozen in liquid nitrogen, pooled into one sample per tank, and stored at −80°C for 8 months until analysis of TRP, 5-HT, and 5-HIAA.

Plasma samples were analyzed for TRP using the Bloxam and Warren modification[9] of the fluorometric method of Denkla and Dewey.[10] Fluorescence was detected by an Aminco-Bowman Spectrofluorometer (American Instrument Company, Silver Spring, MD, USA) with an excitation wavelength of 373 nm and an emission wavelength of 452 nm. For each tank, two of the three pooled plasma samples were examined in duplicate. The mean of these four values was then used as the plasma TRP value for that replicate.

Duplicate samples of approximately 100 mg of frozen pooled brain were homogenized in exactly four times the volume of ice-cold 0.1 M H_2SO_4 using a con-

ical glass tissue grinder (Canadawide Scientific Ltd., Toronto, Canada) in an ice bath. Homogenates were then centrifuged at 16,600 × *g*, 4°C for 15 minutes. The supernatants were aspirated and recentrifuged at 16,600 × *g*, 4°C for a further 15 minutes, after which they were transferred to clean tubes and frozen at −80°C until analysis. Brain TRP, 5-HT, and 5-HIAA were simultaneously assayed by HPLC with electrochemical detection using a modification of the method of Mefford.[11] One hundred microliter aliquots of sample were injected onto a C-18 column (CSC-S ODS2, 25 cm × .46 cm, particle size 5 μm; Chromatography Sciences Company, Inc., Toronto, Canada) using a Waters Associates WISP 710A (Waters Scientific, Mississauga, Ontario, Canada). The column was kept in a water jacket at 16°C using a Forma Scientific refrigerated circulating bath (Model 2376, Mallinckrodt, Inc., Marietta, OH, USA) and a mobile phase flow rate of 0.9 ml/min was maintained using a Waters Associates Model M-6000A pump. Voltages of the electrochemical detector (ESA Coulochem Model 5100A, SPE Limited, Rexdale, Ontario, Canada) were set at 0.35 V (detector 1) and 0.70 V (detector 2). The mobile phase consisted of 0.1 M citric acid, 0.1 M sodium acetate, 0.043 mM sodium octyl sulphate in a dilution 1:9 (vol/vol) of HPLC grade methanol and glass distilled water.

Statistical analysis

Body weight and feed intake data were subjected to analysis of variance with significance of differences tested at the 5% level using Tukey's Honestly Significant Difference procedure.[12] Linear regression was used to determine the effect of dietary level of TRP on plasma TRP, brain TRP, brain 5-HT, and brain 5-HIAA. Significance of differences from zero slope were tested at the 0.01% level using a Student's *t* test.[13]

Results

After 14 weeks of feeding the test diets, body weight was significantly lower in fish receiving diets containing 0.06%, 0.16%, and 0.21% TRP than in those fed higher TRP levels. However, supplementation above 0.26% did not further improve growth (*Table 2*). Feed intake was also significantly lower in fish fed the diets containing 0.06% and 0.16% TRP. There were no differences in feed intake in fish fed the diets containing 0.21% or more TRP (*Table 2*).

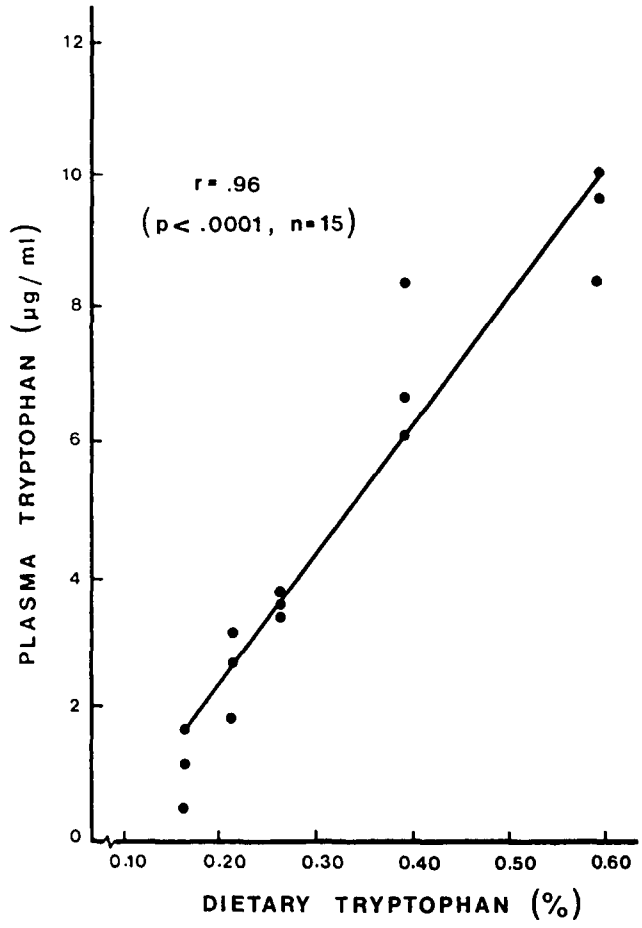

Figure 1 Effect of dietary TRP level on plasma TRP in juvenile rainbow trout (*S. gairdneri*) raised for 16 weeks at 15°C.

Plasma TRP in the fish increased significantly with increasing dietary TRP level (*Figure 1*). Similarly, brain TRP was also significantly elevated by increased dietary levels of TRP (*Figure 2*), as were 5-HT (*Figure 3*) and its metabolite, 5-HIAA (*Figure 4*). The ratios of brain 5-HT to 5-HIAA were not affected by

Table 2 Effect of feeding diets containing different tryptophan levels for 14 weeks at 15°C on body weight and feed intake in juvenile rainbow trout (*Salmo gairdneri*)

	Diet (tryptophan level) (%)					
	1 (0.06)	2 (0.16)	3 (0.21)	4 (0.26)	5 (0.39)	6 (0.59)
Initial body weight*	3.4^{a} (±0.07)	3.4^{a} (±0.06)	3.5^{a} (±0.03)	3.4^{a} (±0.03)	3.3^{a} (±0.03)	3.4^{a} (±0.03)
Final body weight*	3.8^{k} (±0.05)	13.7^{l} (±0.12)	31.7^{m} (±0.66)	36.6^{n} (±0.76)	37.6^{n} (±0.42)	36.5^{n} (±0.58)
Total feed intake*	4.3^{x} (±0.10)	13.1^{y} (±0.15)	28.9^{z} (±0.24)	29.6^{z} (±0.16)	29.6^{z} (±0.17)	29.6^{z} (±0.18)

Results are the means of three replicates (± SEM). Values within a row sharing a common superscript are not significantly different ($P < .05$).

* Body weight is expressed as g/fish, wet matter basis. Feed intake is expressed as g/fish, dry matter basis.

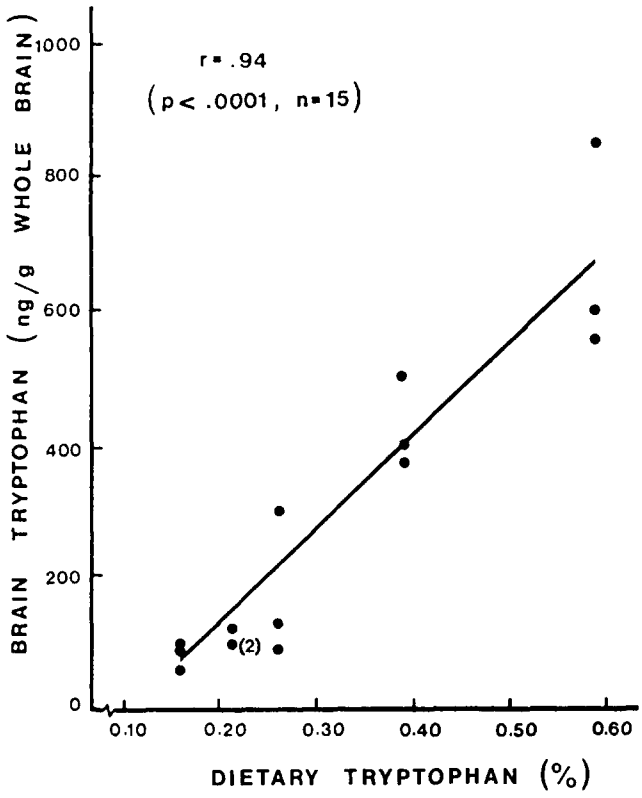

Figure 2 Effect of dietary TRP level on brain TRP in juvenile rainbow trout (*S. gairdneri*) raised for 16 weeks at 15°C.

the dietary treatments and were as follows (expressed as mean with SEM in parentheses): diet 2, 4.3 (1.19); diet 3, 8.8 (2.66); diet 4, 7.9 (0.93); diet 5, 3.7 (0.59); and diet 6, 3.9 (0.09).

Discussion

In rainbow trout, as in most other species of animals, TRP is a dietary essential amino acid. Therefore, at dietary levels of TRP that were below requirement, feed intake and body weight gain were impaired, indicating inadequate TRP for maximal tissue deposition.

As is seen in mammals, increased dietary TRP levels in these fish elevated plasma total TRP. Because the dietary levels of the other large neutral amino acids (LNAA, which compete with TRP for transport across the blood-brain barrier) were kept constant, this effectively increased the plasma TRP to LNAA ratio, resulting in increased brain levels of TRP. Furthermore, as is also found in mammalian species, these increased brain TRP levels caused elevations in brain 5-HT and 5-HIAA. It is also interesting to note, however, that over the sevenfold increase in brain TRP noted in this study, brain 5-HT and 5-HIAA levels paralleled one another, both continuing to rise in a linear fashion; this may suggest that the enzyme TRP 5-monooxygenase is not saturated even at the highest levels of brain TRP. While this might indicate that the kinetics of trout TRP 5-monooxygenase are somewhat different from those of mammals, direct studies of the enzyme itself would be helpful in determining whether this is, indeed, the case.

The increases in brain 5-HT and 5-HIAA resulting from increased dietary TRP levels are of interest in fish, as it has been shown by other investigators that brain or cerebrospinal fluid 5-HT and 5-HIAA can also be altered by stressors such as spawning and water salinity changes and by the water-borne insecticide, methoxychlor.[14–16] Furthermore, Pouliot et al.[4] have demonstrated that hypoxia-induced changes in brain 5-HT and 5-HIAA are different in fish fed diets in which the proteins differ in their amino acid spectra. In their experiments, fish fed a commercial fish meal-based diet showed decreased hypothalamic 5-HIAA when exposed to hypoxia, while fish fed either a poultry by-product or a vegetable meal-based diet showed no change in hypothalamic 5-HIAA. Based on these experiments and on our results from this study, it would be interesting to determine if varying the TRP supply in the diet could protect brain serotonergic status, thus minimizing some of the deleterious effects of some stressors.

In mammals, increases in brain 5-HT and 5-HIAA are believed to result in a decreased appetite in general, a decrease in carbohydrate appetite, or, specifi-

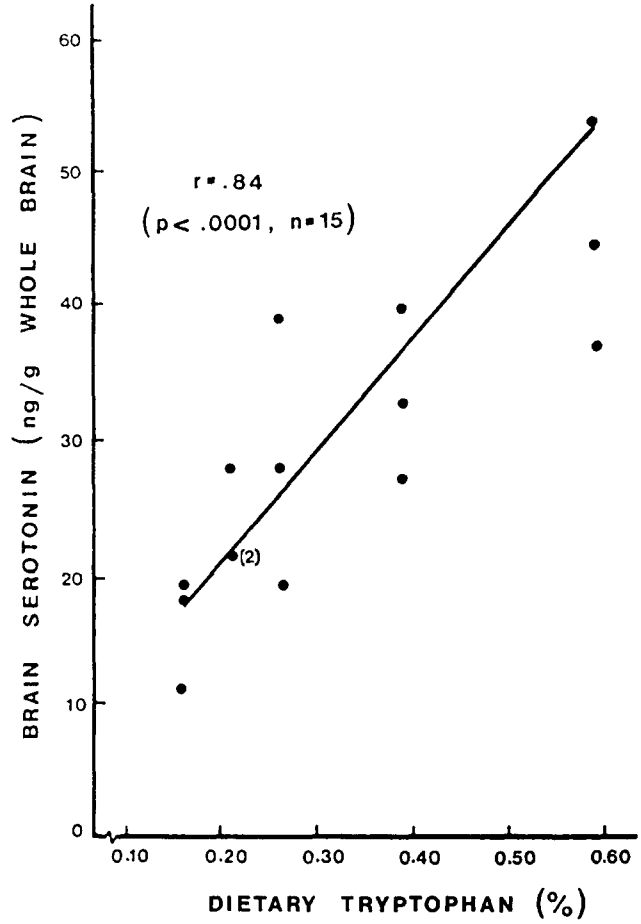

Figure 3 Effect of dietary TRP level on brain serotonin in juvenile rainbow trout (*S. gairdneri*) raised for 16 weeks at 15°C.

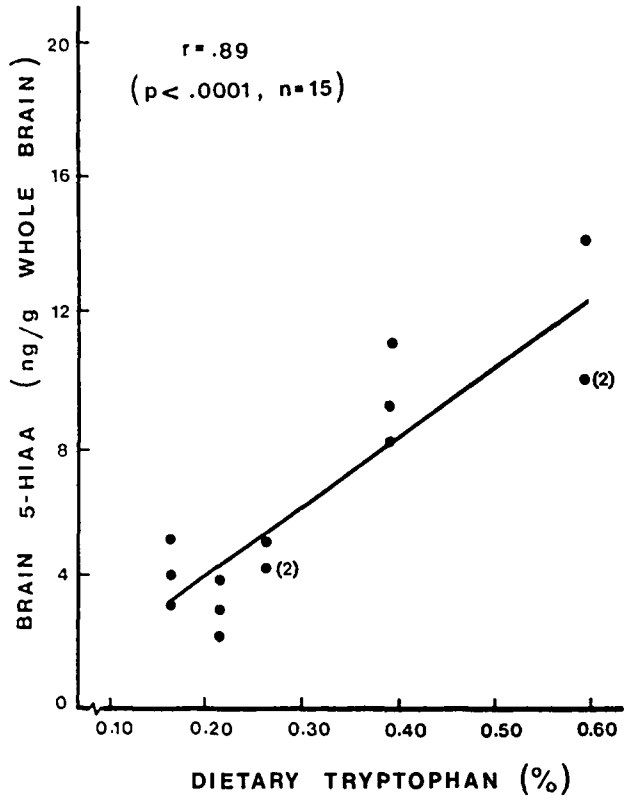

Figure 4 Effect of dietary TRP level on brain 5-HIAA in juvenile rainbow trout (*S. gairdneri*) raised for 16 weeks at 15°C.

cally, a decrease in the preference for the carbohydrate versus the protein fraction of a meal.[2,3] Therefore, in this study, it was somewhat surprising to find that while brain 5-HT and 5-HIAA continued to rise in response to increases in dietary TRP even above the TRP requirement level of 0.25%,[5] this was not reflected in increased feed intake and weight gain. This might be explained by a number of factors. For example, it is possible that the serotonergic satiety mechanism operates in trout, but due to chronic ingestion of these diets, postsynaptic 5-HT receptor down-regulation occurred, resulting in a buffering of the behavioral response (satiety) which would otherwise have resulted from these increases in 5-HT synthesis and release into the synapse. Another possibility is that the brain serotonergic system, rather than acting as a general satiety agent, acts primarily to decrease carbohydrate consumption. In this experiment, the dietary carbohydrate was entirely raw corn starch. However, Cho and Slinger have shown both raw and autoclaved corn starch to be poorly digested by rainbow trout, with apparent digestibility estimates for raw corn starch of 0% to 13%.[17,18] Therefore, in these diets, as in the natural diet of the carnivorous rainbow trout, the actual level of digestible carbohydrate is extremely low. If the serotonergic satiety system does, in fact, control only carbohydrate appetite, this very low level of digestible carbohydrate may have precluded the operation of this system. Furthermore, if, in fact, serotonin alters only the relative preference for carbohydrate versus protein rather than controlling carbohydrate appetite per se, a different experimental approach that included diets with varying carbohydrate to protein ratios would be required.

While these explanations are possible, given the very low carbohydrate intake in the natural diet of trout, another possibility is that serotonergic neurons never play a physiologic role in appetite control in rainbow trout. In this case, fish would not be expected to respond to differences in brain serotonin status by altering their selection of diets with varying carbohydrate to protein ratios. Instead, other behaviors, such as spawning, locomotor activity, aggression, sleep, adaptation to salinity changes, etc., which may be regulated by serotonergic neurons, might be modified by these alterations in brain 5-HT synthesis and release.

In this study, we have demonstrated that in rainbow trout, increased dietary TRP levels result in increased plasma TRP, brain TRP, brain 5-HT, and brain 5-HIAA. Current studies are now aimed at understanding aspects of the physiologic relevance of changes in brain serotonergic status in trout.

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